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CONTINUOUS ANIMAL EXPOSURE TO A MIXTURE OF DICHLOROMETHANE AND 1,1,1-TRICHLOROETHANE

FINAL REPORT

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Continuous Animal Exposure to a Mixture of Dichloromethane and 1, 1, 1-Trichloroethane

An investigation of the effects of combined exposure of animals to dichloromethane and 1, 1, 1-trichloroethane was conducted using atmospheric concentrations of each solvent which had individually produced minimal measureable effects on livers. This study was undertaken at the request of the National Aeronautic and Space Administration to determine if previously established spacecraft TLV's (threshold limit values) for the individual solvent compounds were valid when both were present in an astronaut's breathing environment under continuous exposure conditions.

Exposure concentrations selected for this study were based on the results of 100-day continuous exposures to various animal species as reported by MacEwen et al., 1972, Haun et al., 1972, Weinstein et al., 1972 and MacEwen and Vernot, 1973 in which concentrations of 100 ppm dichloromethane and 1000 ppm 1, 1, 1-trichloroethane independently produced comparable degrees of liver triglyceride levels and fat accumulation in livers of mice.

Two groups of animals consisting of 4 Rhesus monkeys, 8 beagle dogs, 40 Sprague-Dawley CFE rats and 180 ICR - CF-1 mice were housed in Thomas Dome exposure chambers where one group served as controls for the experimental group.

All dogs, monkeys and rats weighed on a biweekly schedule at which time blood samples were drawn from the dogs and monkeys for hematology and clinical chemistry determinations listed below:

HCT	Sodium	Glucose
HGB	Potassium	Alkaline Phosphatase
RBC	Calcium	SGPT
WBC	Albumin	SGOT
Blood Indices	Total Protein	

Subgroups of 10 mice each were serially sacrificed on a weekly schedule from both control and exposed groups for gross pathologic examination, body weight, liver weight, and liver triglyceride determinations. Histologic preparations were also examined for liver fat content. These samples were taken throughout the exposure period of 13 weeks and for 2 additional postexposure weeks to determine reversibility of any observed effects.

At the conclusion of the exposure period all rats and monkeys were necropsied for complete histopathologic examination as were most of the dogs. Two control and 2 exposed dogs were held one month post-exposure, again for the purpose of determining reversibility of hematologic or clinical chemistry changes.

During the 90-day experimental period the animals were fed ad libitum at daily intervals with the remaining food discarded and replaced to minimize solvent adsorption and subsequent oral intake by the animals.

The solvents used in animal exposures were technical grade obtained in 55 gallon drums. Both the dichloromethane and the 1,1,1-trichloroethane were pumped from the drums into a heated air duct and the resulting vaporized mixture was then introduced into the chamber air supply duct. The chamber concentrations were continuously monitored to permit control by adjusting the speed of the liquid transfer pumps.

Although chamber concentrations of each solvent had been satisfactorily analyzed with a flame ionization hydrocarbon analyzer in the independent studies, the hydrocarbon analyzer could not be used for this comparative study since it could not differentiate between the 2 compounds. Since the solvents are relatively unreactive and had good

infrared spectra, 2 single beam IR analyzers with variable wavelength and pathlengths (Miran I[3]) were obtained for this purpose. Noninterfering absorbance bands were selected for each compound from full range infrared scans. Air samples were drawn from either the control or exposure chamber through a 3-way valve and passed through the 2 IR analyzers in series determining the concentration of each solvent in sequence.

The solvent introduction and analysis systems are shown in Figure 1. The air sampled from the control animal chamber was utilized to set the zero baseline for the analyzers. The instruments were calibrated daily against precisely measured standards mixed in gas bags which spanned the expected range of each solvent. The specific instrument conditions used are listed in Table 1. The nominal air concentration selected for each solvent was 100 ppm of dichloromethane and 1000 ppm of 1, 1, 1-trichloroethane. The actual mean concentration for the 90-day exposure period was 99.6 ppm dichloromethane and 993 ppm 1, 1, 1-trichloromethane. No fluctuations of solvent concentrations in the exposure chamber exceeded 10% of the preselected level during the course of the study.

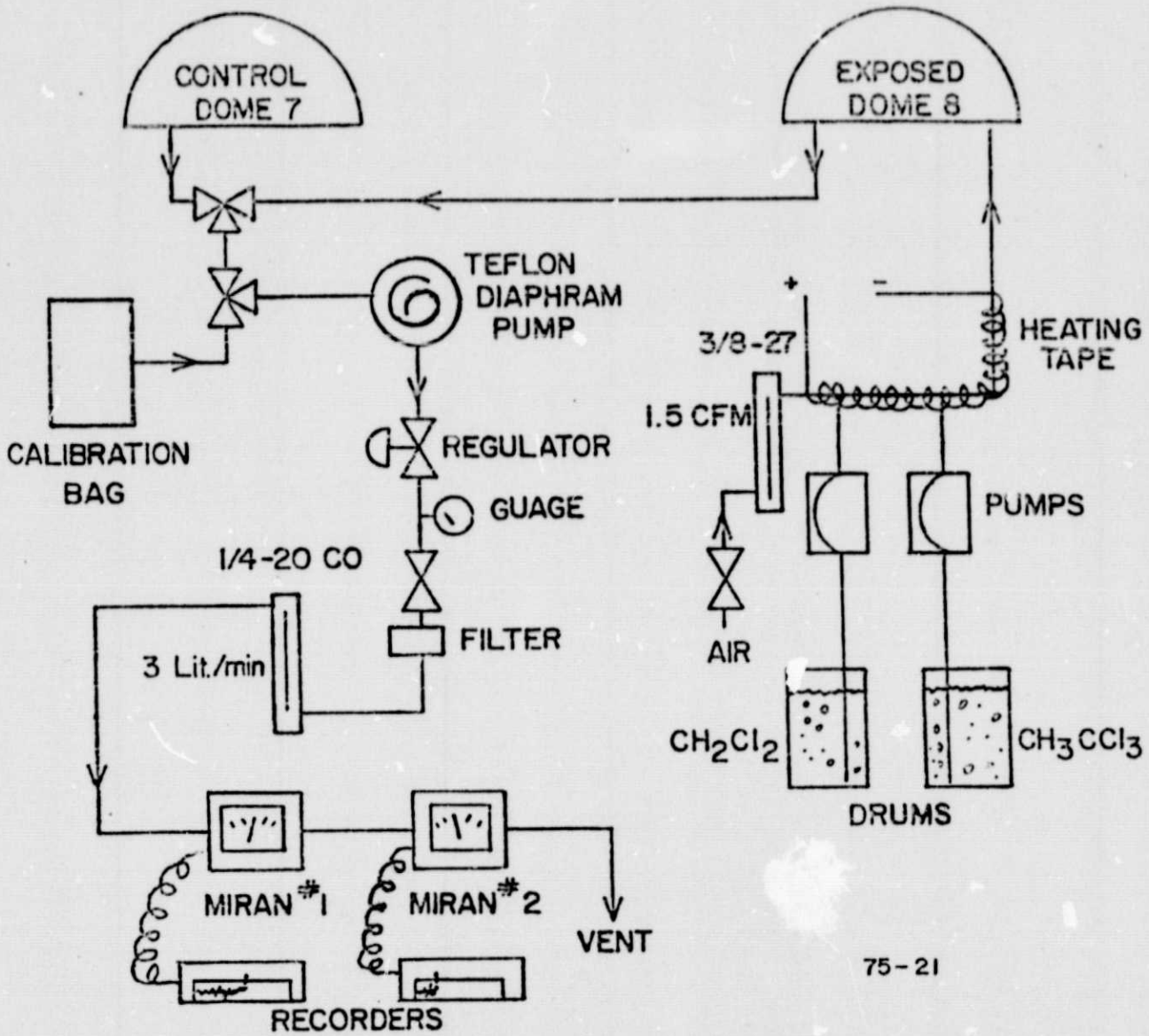

Figure 1. Mixed solvent inhalation chamber contaminant introduction and monitoring systems.

TABLE 1. STANDARD INSTRUMENTAL SETTINGS FOR CONTINUOUS IR MONITORING OF DICHLOROMETHANE AND 1,1,1-TRICHLOROETHANE

Control Control	IR Analyzer-I (Dichloromethane)	IR Analyzer-II (1,1,1-Trichloroethane)
Slit Width	0.50 mm	0.50 mm
Wavelength	7.78μ	13.8μ
Pathlength	6.75 m	0.5 m
Absorbance	0.1 A	1 A
Gain	10 X	10 X
Time Constant	1	10
Cut Off Frequency	-	0.05

The growth rate of rats was not significantly affected by exposure to the solvent mixture as shown in Figure 2 and in mice, a significant difference between control and exposed group weight was only seen at the sixth week of the experimental period (Table 7). The difference between test and control groups, although not statistically significant was also greatest at the six week point for the rats as well as for dogs and monkeys as shown in Table 2.

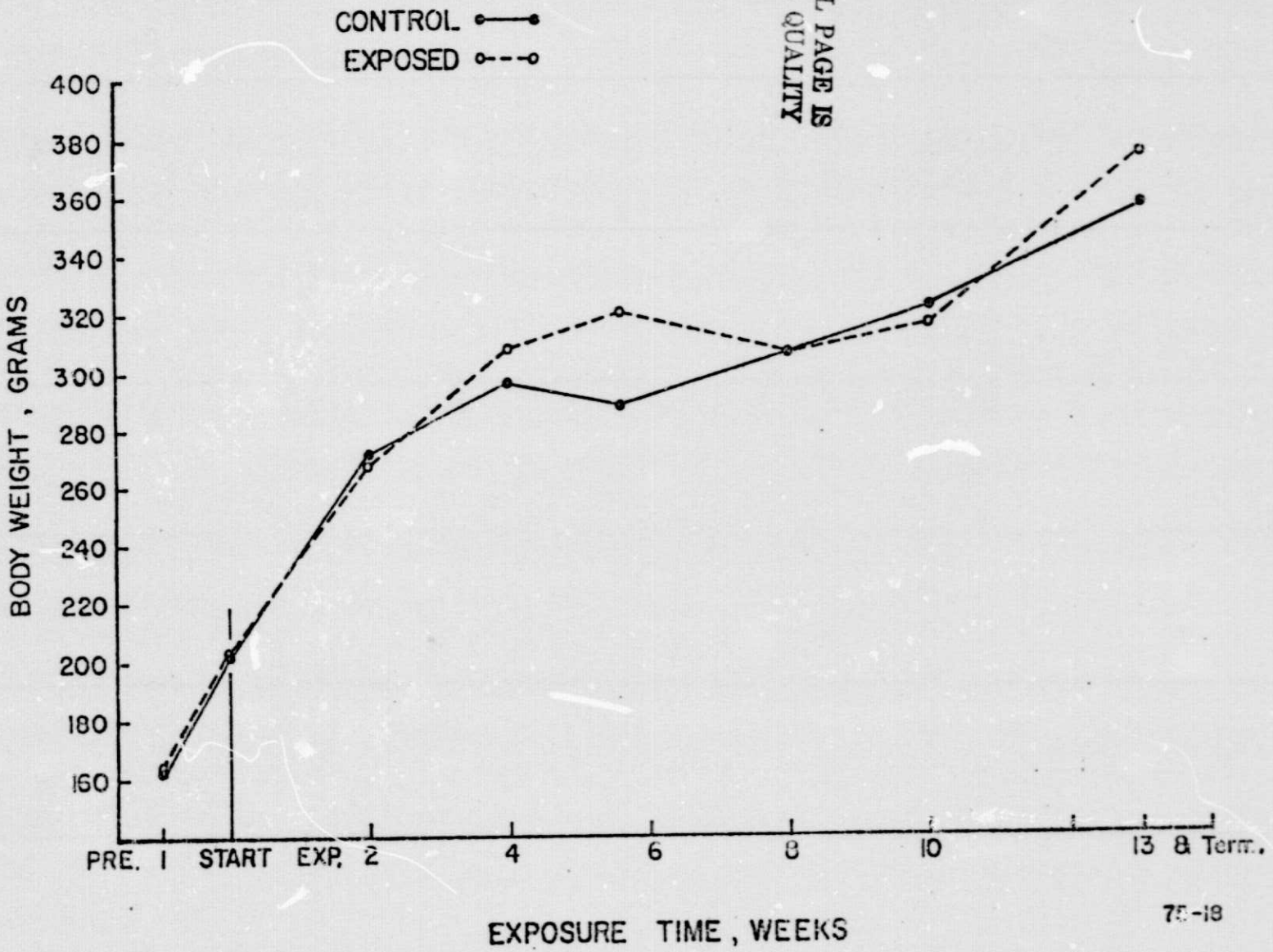

Figure 2. Effect of continuous exposure to dichloromethane and 1, 1, 1-trichloroethane on

TABLE 2. EFFECT OF CONTINUOUS MIXED SOLVENT INHALATION EXPOSURE ON DOG AND MONKEY BODY WEIGHTS

(Mean Body Weight in Kilograms)

Exposure Length Weeks	Dogs (N = 8)		Monkeys (N = 4)	
	Control	Exposed	Control	Exposed
-2	8.90	8.64	3.51	3.42
0	9.09	8.68	3.30	3.30
2	10.05	9.45	3.53	3.35
4	10.36	9.57	3.62	3.67
6	10.69	9.60	3.78	3.49
8	10.89	9.72	3.81	3.61
10	10.63	9.78	3.82	3.75
13	10.77	10.18	3.65	3.49

Clinical chemistry values for monkeys (Table 3) show no significant differences between test animals and their air exposed controls. Dogs and monkeys, however, showed slightly increased hematocrit, red cell counts and hemoglobin values during exposure to the mixed solvents (Tables 4 through 6). These changes were accompanied by a slight rise in reticulocyte counts and a slight decrease in mean corpuscular hemoglobin (MCH). These findings are consistent with the finding of increased carboxyhemoglobin levels in exposed dogs and monkeys. The mean carboxyhemoglobin value in dogs was 0.9% saturation and in monkeys was 1.2%. These results are slightly lower than that reported for dichloromethane alone (MacEwen et al., 1972) and may represent a lower metabolic conversion of dichloromethane to carbon monoxide in the presence of 1, 1, 1-trichloroethane.

Dogs exposed to the mixture of solvents had a significant increase in serum alkaline phosphatase and a decrease in serum glutamic oxaloacetic transaminase (SGOT) both of which returned to control levels during the postexposure observation. The biological significance of these changes is difficult to access since the decreased SGOT values were still within the normal range and although the increase in alkaline phosphatase may indicate a mild hepatotoxic response, there were no other changes observed in dogs to support such a conclusion.

TABLE 3. MEAN CLINICAL CHEMISTRY VALUES FOR MONKEYS CONTINUOUSLY EXPOSED FOR 90 DAYS TO A MIXTURE OF 100 PPM DICHLOROMETHANE AND 1000 PPM 1,1,1-TRICHLOROETHANE

Weeks	Sodium (MEq/L)		Potassium (MEq/L)		Calcium (mg %)		Albumin (g %)		Total Protein (g %)	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure										
-4	153	156	3.9	4.3	9.9	16.1	4.7	4.6	7.9	7.9
-0	150	152	4.1	4.6	10.0	10.1	4.6	4.8	7.8	8.1
Exposure										
2	149	151	4.9	5.9	10.5	10.6	4.4	4.2	7.7	7.8
4	156	154	4.8	5.1	11.4	11.1	4.7	4.8	8.1	8.3
6	156	157	5.1	5.2	11.4	11.3	4.3	4.4	7.6	8.0
8	155	158	5.2	5.3	11.4	11.3	4.4	4.2	7.8	7.7
10	154	155	4.6	5.4	11.2	11.7	4.5	4.7	7.9	8.1
13	158	156	5.0	5.5	11.5	11.3	4.9	5.0	8.6	8.4

TABLE 3. CONTINUED

Weeks	Glucose (mg %)		Alk. Phos. (KA)		SGPT (RF)		SGOT (Karl Units)	
	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure								
-4	79	91	37	60	35	37	33	36
-0	87	74	46	58	36	41	34	45
Exposure								
2	101	93	28	41	32	38	30	30
4	112	101	30	41	35	37	37	35
6	101	121	29	42	37	36	28	37
8	93	116	30	36	33	30	24	34
10	99	105	30	37	32	33	32	34
13	94	114	37	36	32	48	33	56

TABLE 4. MEAN HEMATOLOGY VALUES FOR MONKEYS CONTINUOUSLY EXPOSED FOR 90 DAYS TO A MIXTURE OF 100 PPM DICHLOROMETHANE AND 1000 PPM 1,1,1-TRICHLOROETHANE

Weeks	HCT (vol %)		RBC (millions)		HGB (g %)		WBC (cc)	
	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure								
-4	40	41	5.5	5.5	13.2	13.7	7.6	11.3
-0	39	40	5.6	5.6	13.2	13.6	8.7	13.0
Exposure								
2	43	40	5.7	5.8	13.8	13.2	9.4	14.9
4	41	40	5.4	5.6	13.1	13.2	12.9	12.1
6	43	40	5.7	5.6	13.7	13.3	11.5	13.2
8	42	38	5.8	5.5	13.7	12.7	11.0	14.8
10	42	40	5.6	5.6	13.6	13.2	7.9	12.4
13	42	40	5.8	5.8	13.4	13.7	10.1	9.2

TABLE 4. CONTINUED

Weeks	Reticulocyte (%)		MCV (cm)		MCH (uug)		MCHC (%)	
	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure								
-4	0.9	0.6	72.9	76.1	24.1	25.2	33.1	33.1
-0	0.6	1.0	71.8	72.2	24.2	24.5	33.7	34.0
Exposure								
2	0.6	0.7	75.1	69.4	24.3	22.8	32.4	32.8
4	1.2	0.9	76.1	71.3	24.6	23.4	32.3	32.9
6	0.8	1.3	74.4	71.7	23.9	24.0	32.1	33.5
8	1.2	0.6	72.5	69.6	23.6	23.0	32.6	33.1
10	0.7	1.1	74.1	71.9	24.3	23.7	32.8	33.1
13	1.1	1.8	71.8	68.1	23.2	22.8	32.3	33.4

TABLE 5. MEAN CLINICAL CHEMISTRY VALUES FOR DOGS CONTINUOUSLY EXPOSED FOR 90 DAYS TO A MIXTURE OF 100 PPM DICHLOROMETHANE AND 1000 PPM 1,1,1-TRICHLOROETHANE

Weeks	Sodium (MEq/L)		Potassium (MEq/L)		Calcium (mg %)		Albumin (g %)		Total Protein (g %)	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure										
-4	149	150	4.8	4.8	10.2	9.8	3.1	2.9	6.2	6.1
-0	147	147	4.5	4.7	9.8	9.5	3.2	3.0	6.3	6.1
Exposure										
2	148	150	5.0	4.9	10.0	9.4	2.8	2.5	6.3	6.0
4	151	150	5.1	5.3	10.0	9.9	3.1	3.0	6.6	6.3
6	154	152	5.2	5.0	11.0	10.5	3.0	2.8	6.2	6.1
8	150	152	4.9	5.0	10.6	10.4	3.0	2.7	6.4	6.2
10	150	148	4.9	5.0	10.8	10.2	3.2	3.0	6.7	6.4
13	152	151	5.1	4.8	11.0	10.3	3.2	3.2	6.9	6.6
Post-Exposure										
2	154	152	4.8	5.2	10.8	10.2	3.5	3.1	6.6	6.4
5	153	155	4.6	5.1	10.5	10.1	3.6	3.1	6.7	6.4

TABLE 5. CONTINUED

Weeks	Glucose (mg %)		Alk. Phos. (KA)		SGPT (RF)		SGOT (Int'l Units)	
	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure								
-4	108	106	4 5	4.6	31	29	--	--
-0	105	103	4.6	4.4	33	31	38	41
Exposure								
2	82	94	5.9	4.7	33	37	20**	36
4	97	105	6.1**	4.2	32	38	16**	36
6	90	107	6.8**	4.4	28	36	16**	37
8	105	106	7.7**	4.8	24	28	13**	31
10	104	101	7.3**	4.5	27	31	16**	38
13	115	118	7.5**	3.9	27	32	15**	29
Post-Exposure								
2	110	89	5.8	3.8	28	31	21	28
5	121	109	4.0	3.9	20	25	25	31

TABLE 6. MEAN HEMATOLOGY VALUES FOR DOGS CONTINUOUSLY EXPOSED FOR 90 DAYS TO A MIXTURE OF 100 PPM DICHLOROMETHANE AND 1000 PPM 1,1,1-TRICHLOROETHANE

Weeks	HCT (vol %)		RBC (millions)		HGB (g %)		WBC (cc)	
	Test	Control	Test	Control	Test	Control	Test	Control
Pre-Exposure								
-4	44	44	6.1	6.1	14.6	15.0	11.0	11.5
-0	41	42	6.0	6.1	14.3	14.4	10.4	12.5
Exposure								
2	47	42	6.5	6.0	15.7	14.4	14.8	15.9
4	47	44	6.7	6.2	15.8	14.9	15.3	14.4
6	47	43	6.8	6.1	15.4	14.7	14.8	14.4
8	46	43	6.9	6.1	15.5	14.5	14.1	13.5
10	48	45	7.2	6.3	16.4	15.5	14.0	13.6
13	47	45	7.1	6.1	16.1	15.4	12.2	12.7
Post Exposure								
2	48	48	5.6	5.9	15.4	15.9	6.7	11.3
5	45	46	6.7	6.6	15.0	15.3	7.4	11.8

TABLE 6. CONTINUED

Weeks	Reticulocyte (%) Test	Reticulocyte (%) Control	MCV (cm) Test	MCV (cm) Control	MCH (uug) Test	MCH (uug) Control	MCHC (%) Test	MCHC (%) Control
Pre-Exposure								
-4	0.7	0.6	72.5	72.7	25.0	24.6	33.2	33.9
-0	0.4	0.4	69.0	68.6	23.9	23.7	34.7	34.5
Exposure								
2	0.8	0.5	72.1	70.3	24.3	24.0	33.7	34.2
4	1.2*	0.7	70.0	71.2	23.7	24.1	33.8	33.8
6	0.9	0.8	69.4	69.9	22.8*	23.9	32.8	34.3
8	0.9	0.6	67.3	70.3	22.8*	23.8	33.8	33.9
10	0.9	0.6	67.1	70.9	22.9**	24.6	34.1	34.8
13	0.8	0.7	66.1	73.8	22.7**	25.3	34.4	34.3
Post-Exposure								
2	0.8	0.6	84.8	81.8	27.5	27.2	32.4	33.1
5	0.4	0.5	65.6	68.9	21.8	23.2	33.2	33.6

Sections of mouse liver from the sacrifice group were stained with Oil-Red-O at weekly intervals during the course of the study. The livers had begun to accumulate some fat droplet in a diffuse pattern after one week of continuous exposure to the solvent mixture.

There was a slow but steady increase in liver fat content during the 13 weeks of exposure reaching a 2.5 + average on a scale of 0 to 4 + by the end of 90 days. Of ten mice held for one week postexposure only one showed any residual trace of fatty deposition and by the second post-exposure week the mouse livers were completely clear of fat with only a mild inflammatory response remaining.

The liver weights of the exposed mice were slightly heavier than those of the controls and the liver to body weight ratios (Table 7) of the exposed mice became significantly higher than that of controls after three weeks. The significance of the difference between exposed and control animals dropped from the 0.01 to 0.05 confidence level by the end of one week postexposure but was not reversed completely by the end of the second postexposure week. Although liver triglyceride values of the exposed mice appeared to be higher than control values, an extremely wide variability in analytical measurements made the results unsuitable for comparative purposes.

TABLE 7. EFFECT OF CONTINUOUS MIXED SOLVENT INHALATION EXPOSURE ON MOUSE GROWTH RATE AND LIVER WEIGHT

Weeks Exposed	Body Weight (grams)		Liver Weight (grams)		Liver/Body Weight	
	Test	Control	Test	Control	Test	Control
1	23.1	24.2	1.3	1.1	.049	.054
2	24.9	27.9	1.5	1.5	.058	.054
3	26.4	30.1	1.5	1.5	.057*	.050
4	27.6	30.4	1.5	1.5	.055*	.049
5	29.2	30.4	1.6	1.6	.054	.051
6	28.3*	31.2	1.5	1.5	.052	.047
7	30.4	31.6	1.8	1.5	.058**	.048
8	29.3	31.2	1.7	1.7	.058	.054
9	31.4	32.6	1.7	1.6	.054**	.047
10	30.2	35.0	2.0	1.7	.065**	.048
11	32.1	31.8	2.0**	1.4	.062**	.045
12	32.0	31.9	1.9*	1.6	.059**	.049
13	30.3	33.7	1.7	1.4	.046**	.042
Post-Exposure						
1	32.2	33.5	1.7	1.5	.053*	.045
2	31.3	34.5	1.6	1.4	.051*	.040

Rat organ weight measurements, shown in Table 8, taken at the conclusion of the 90-day exposure period exhibited an increase in liver; spleen and kidney weights and organ to body weight ratios of exposed animals of which the splenic gross weight and organ to body weight splenic ratio reached the 0.05 significance level.

Tissues for transmission electron microscopy (TEM) were prepared using livers from mice after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 weeks of exposure and 1 and 2 weeks of recovery; i.e., weeks 14 and 15. Samples from 10 control and 10 treated animals for each week of exposure have been fixed and embedded. Preliminary observations of 1μ sections by light microscopy (LM) and of thin sections by TEM have been completed on 2 representative specimens each from 6 of the 15 weekly exposure and recovery periods. Evaluation of specimens from the remaining 9 weekly periods will require an additional 6-9 months. Sampling procedures and technical methods were as described for the exposure to single solvents. Thus while TEM, by its nature is slow, the study is progressing satisfactorily considering the lack of full-time faculty on location who were present for evaluation of previous solvent studies.

TABLE 8. EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO A MIXTURE OF 100 PPM DICHLOROMETHANE AND 1000 PPM 1,1,1-TRICHLORCETHANE ON RAT ORGAN WEIGHTS

	Mean Organ Weight* (grams)		Mean Organ/Body Weight Ratio**	
	Exposed	Control	Exposed	Control
Heart	1.26	1.27	0.363	0.373
Lung	2.57	2.44	0 751	0.720
Liver	11.0	10.2	3.174	2.968
Spleen	0.80**	0.71	0.230**	0.205
Kidney	2.74	2.55	0.788	0.744

* N = 20

** Different from the control mean at the 0.05 significance level.

In the animals examined to date, all of the lesions described by LM of 1μ sections and by TEM in livers from animals exposed to either dichloromethane or trichloroethane singularly have been observed. Specifically, these consist of increases in numbers and variation of size of triglyceride droplets, osmiophilic membraneous whorls ("myelin figures") most commonly in lysosomal vesicles, increase agranular (smooth) endoplasmic reticulum, increase in microbodies (peroxisomes), and dilation or vaculation of the granular (rough) endoplasmic reticulum with loss of ribosomes and polyribosomes. The severity of the lesions varies from cell to cell in exposed animals and similar, but much less severe, changes are occasionally seen in livers from control animals. As additional livers from other exposure periods are examined, an attempt will be made to determine the sequence of lesions and to correlate that information with both duration of exposure and location within the hepatic lobule. The sequence, severity and localization of lesions in animals exposed to mixed solvents will be compared to those from animals exposed to the solvents singularly.

At necropsy there were no apparent differences between the control and mixed solvent exposed dogs. The finding was confirmed by complete histologic examination of selected tissues from heart, lung, brain, liver, spleen, kidney, adrenals and testes.

In monkeys only one animal exposed to the solvent mixture showed a slight increase in the amount of fat deposited in hepatocytes of the periacinal region. In other monkeys the only lesion observed was that produced by lung mites in both exposed and control groups.

The common pathologic finding in both control and exposed rat groups was chronic respiratory disease (CRD) ranging from a mild to moderate state in those animals necropsied at the termination of the 90-day study. One exposed rat that died during the 6th week of the study had increased fat deposition in the periacinal region rated at 3+ on a 0 to 4+ scale. Most exposed rats had scattered areas of renal tubular dilatation containing a pink staining amorphous material. This effect was seen in a few control rats. Oil-Red-O stains of liver tissue were negative for fat.

The only significant finding in this study was the difference between control and exposed mice. There was a consistent finding in the liver tissue of exposed mice of multifocal periacinal areas in which there was vacuolization of surrounding hepatocytes. There were increased amounts of fat in these periacinal areas demonstrated with Oil-Red-O stain. This effect was demonstrated to be reversible and cleared up within 14 days postexposure.

The combined effect of 90-day continuous exposure of animals to 100 ppm dichloromethane and 1000 ppm 1, 1, 1-trichloroethane does not appear to be greater than the effect of each alone. While the exposed mouse livers appeared to contain slightly more fat the degree of increased liver weight and the liver to body weight ratios are slightly lower than those measured for each solvent alone. The metabolic conversion of dichloromethane to carbon monoxide with subsequent carboxyhemoglobin formation and accompanying hematologic changes is also slightly lower in the mixed exposures than was observed in studies with dichloromethane alone. Thus, the spacecraft TLV's for these two common industrial solvents appear to have been set at a safe level for either alone or the combination of both.

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